



Food and feed-related pathogen and toxin binders for an improved gut health

Petra M. Becker*, Piet G. van Wikselaar

Goal

Activated carbon is commonly used in the treatment of diarrhea and for detoxification purposes, because of its high absorption capacity. However, activated carbon does not discriminate between beneficial and harmful compounds and cells. Hence, one of the objectives of this study was to find dietary fiber-related, specific binders for enteropathogens (Fig. 1) and toxins to promote gut health by their egestion. To study the adhesive capacity of different food and feed ingredients, miniaturized adhesion tests were developed for bacterial cells and AB5 toxins, such as the diarrhea-causing *E. coli* heat-labile toxin LT and cholera toxin.

Results and Discussion

With growth as measurand for bacterial adhesion, a simple, high-throughput method was developed for the screening of huge numbers of different binding matrices and bacterial species. The screening of different food and feed components for adhesion of bacteria resulted in highly discriminating product rankings.

Konjac gum, for example, was a good binding matrix for *Salmonella* strains, the pig pathogen *E. coli* K88ac adhered well to tempeh, and the calf pathogen *E. coli* K99 to coffee grounds (Table 1) (Becker and Galletti, 2008).

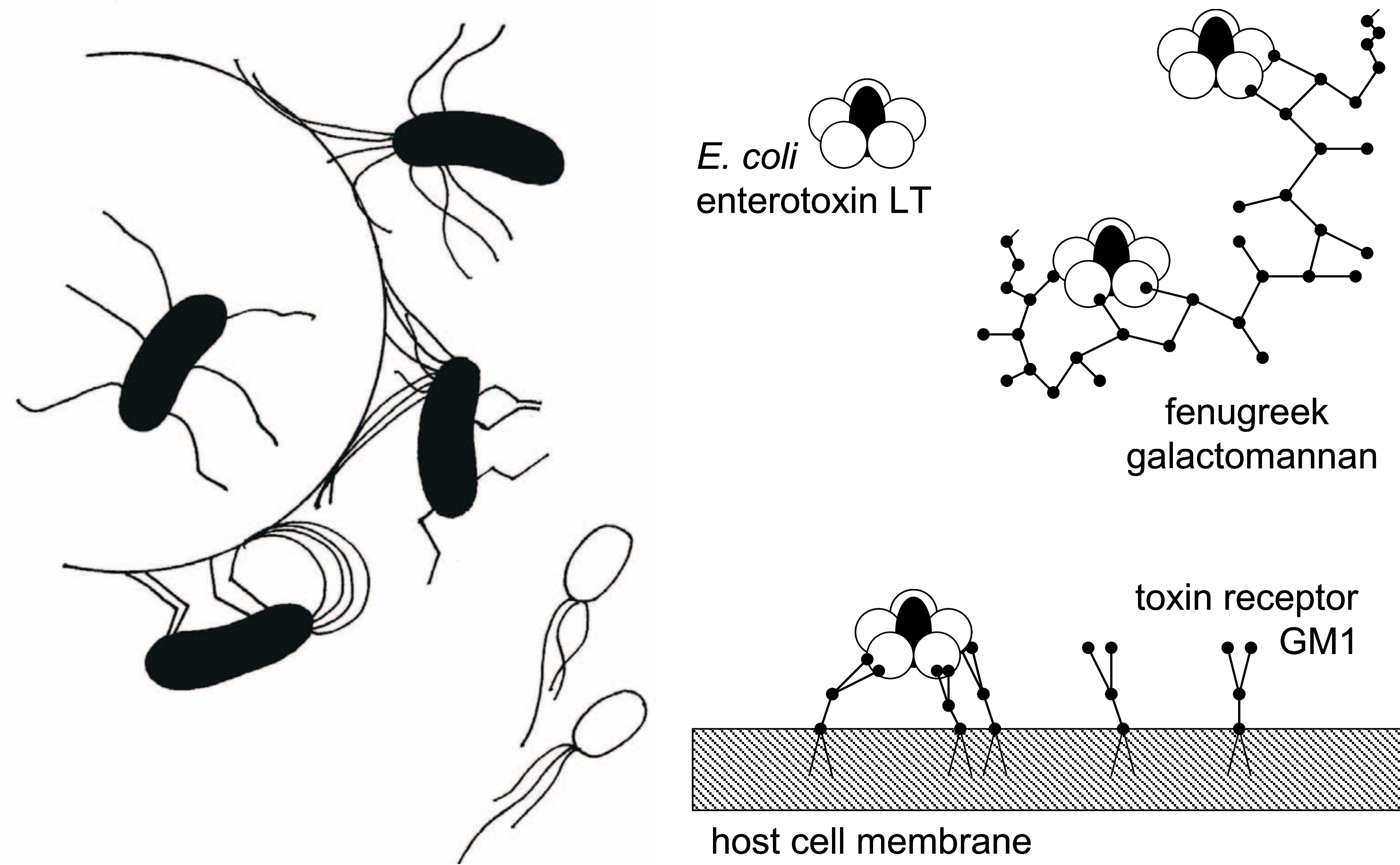


Fig. 1. Provision of alternative adhesion sites for enteropathogenic bacteria to prevent their adhesion to the gut epithelium (Becker, 2005)

Fig. 2. Proposed competitive binding of the *E. coli* heat-labile enterotoxin LT at its GM1-binding sites by fenugreek galactomannan

Methods

The binding capacity of natural substances for bacterial cells was tested by allowing bacteria to adhere to different fibrous materials supplied as well coatings in microtitration plates. The amount of bacteria retained on the materials was determined in an automated way as growth after addition of liquid medium. The test principle was based on an inverse relationship between initial cell densities and the appearance of growth: The higher adhering cell numbers are, the shorter are the detection times of growth (Becker et al., 2007; Becker and Galletti, 2008). The interfering efficiency of natural substances with binding of the diarrhea-causing LT toxin and cholera toxin to the host receptor gangliosid GM1 was tested using an adapted GM1-coated microtiter-well ELISA (Becker et al., 2010).

Host receptor binding of LT and cholera toxin was most efficiently counteracted by skim milk powder and ground fenugreek seed (Fig. 2) (Becker et al., 2010). Employing the adhesion tests, we were also able to show that pea hulls bind *E. coli* K88ac and faba bean hulls bind the ETEC's toxin LT, after a small-intestinal segment perfusion experiment with ETEC K88ac-challenged piglets had indicated that both pea and bean hulls have the potential for successful application in diarrhea prophylaxis and treatment (Becker et al., 2012).

Table 1. Detection times of growth [h] of different *E. coli* and *Salmonella enterica* strains as measure of their adhesion. Products with shortest detection times bound most bacterial cells (Becker and Galletti, 2008).

Product	<i>E. coli</i> K88ac O149:K91	<i>E. coli</i> K99 O9:K35	<i>E. coli</i> O6:K- ATCC 25 922	<i>S. enterica</i> sv. Typhimurium RMM 1287	<i>S. enterica</i> sv. Typhimurium ATCC 13311	<i>S. enterica</i> sv. Enteritidis ATCC 13076
BSA (reference)	10.38 ^{d,e}	8.6 ^{c,d}	5.14 ^h	3.95 ^d	5.33 ^f	4.58 ^b
Artichoke pulp	9.51 ^c	7.49 ^a	2.86 ^{b,c}	3.68 ^b	3.97 ^{b,c}	4.64 ^{b,c}
Carrot pulp	10.43 ^{d,e}	9.13 ^e	3.23 ^d	4.13 ^e	4.48 ^e	4.82 ^{c,e}
Coffee grounds	9.83 ^{c,d}	7.12 ^a	4.20 ^g	4.16 ^e	4.64 ^e	5.11 ^{f,g}
Konjac gum	10.81 ^e	9.19 ^e	2.90 ^c	3.41 ^a	3.56 ^a	3.81 ^a
Locust bean gum	9.93 ^{c,d}	8.63 ^{b,d}	3.51 ^f	4.92 ^f	5.40 ^f	5.00 ^{e,f}
Oil palm kernel meal	8.72 ^b	8.31 ^{b,d}	3.38 ^e	3.34 ^a	3.79 ^b	4.63 ^{b,c}
Pumpkin pulp	7.48 ^a	8.45 ^{b,d}	2.82 ^{a,c}	3.63 ^b	4.24 ^d	5.13 ^{f,g}
Sesame seed	7.74 ^a	8.19 ^b	2.91 ^c	3.40 ^a	3.8 ^b	4.57 ^b
Tempeh (fermented soya)	7.20 ^a	8.56 ^{b,d}	2.76 ^a	3.57 ^b	4.18 ^{c,d}	4.88 ^{d,e}
Tomato pulp	7.79 ^a	8.25 ^{b,c}	2.78 ^{a,b}	3.68 ^b	4.24 ^d	5.28 ^g

Reference

Becker, P.M. (2005) Physiological Achilles' heels of enteropathogenic bacteria in livestock. Curr. Issues Intest. Microbiol. 6, 31-54

Becker, P.M., Galletti, S., Roubos-van den Hil, P., Van Wikselaar, P.G. (2007) Validation of growth as measurand for bacterial adhesion to food and feed ingredients. J. Appl. Microbiol. 103, 2686-2696

Becker, P.M., Galletti, S. (2008) Food and feed components for gut health-promoting adhesion of *E. coli* and *Salmonella enterica*. J. Sci. Food Agricult. 88, 2026-2035

Becker, P.M., Widjaja, H.C.A., van Wikselaar, P.G. (2010) Inhibition of binding of the AB5-type enterotoxins LT-I and cholera toxin to ganglioside GM1 by galactose-rich dietary components. Foodborne Pathog. Dis. 7, 225-233

Becker, P.M., van der Meulen, J., Jansman, A.J.M., van Wikselaar, P.G. (2012) *In vitro* inhibition of ETEC K88 adhesion by pea hulls and of LT enterotoxin-binding by faba bean hulls. J. Anim. Physiol. Anim. Nutr. (in press)